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DURATION OF THE DEVELOPMENT CYCLE OF THE MOSQUITO
VECTORS OF AUTUMN (JAPANESE) ENCEPHALITIS

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The question of the duration of the development cycle in the case of most types of the mosquito vectors of autumn (Japanese) encephalitis virus in the [East Siberian] Maritime Territory has not been studied. There exist only our own researches and the unpublished observations of A.I. LISOVA on *Aedes togoi* Theo. In the 1944 season, we carried out observations on the duration of the development cycle of *Culex tritaeniorhynchus* Giles, *Aedes japonicus* Theo and *Aedes escöensis* Jam.

Working Procedure

Under laboratory conditions, experiments on the duration of the development cycle were conducted both at steady and at varying temperatures. The tests were made in wooden insectaria measuring 80 x 80 cm, the temperature in which could be regulated by means of electric lights of 40-100 candle-power, and also by replacing the plywood side walls with gauze partitions. To avoid any effect of strong light, the cells and water-trays were screened over with several layers of paper. Alimentation conditions for the larvae were maintained at the optimum level. The test insects were allowed to feed on guinea pigs and white mice.

Observations on the duration of the development cycle under natural conditions were made only as regards *C. tritaeniorhynchus*. Both in the laboratory tests and under natural conditions, the air and water temperatures were measured three times a day, namely at 7, 13, and 19 hrs. Data on blood-sucking, on oviposition, hatching, moulting (instars), pupation, and emergence of adults were noted under natural and laboratory conditions.

Lieut. P.M. HERSHSON, Admin. Corps, participated in the laboratory observational work.

I. RESULTS OF THE LABORATORY OBSERVATIONS

1) *O. tritaeniorhynchus*

For the laboratory experiments we used females caught on the premises and also larvae collected from natural bodies of water. We did not succeed in obtaining batches of fertilized eggs from females hatched out in the laboratory. In the experiments, females of this species quite avidly attacked white mice and guinea pigs, particularly when the body surface of the animals was slightly moistened and applied to the darkened cage. One meal of blood was sufficient for the maturation of eggs. We did not obtain eggs from females kept on sugar syrup: here the development of the ovaries was arrested at the second and more rarely in the third phase. We did not observe any relationship between the quantity of blood adsorbed and the number of eggs in a batch. Usually it was the larger females which deposited the greater number of eggs.

Culex tritaeniorhynchus deposits its eggs in long rafts on the surface of the water; the average number of eggs in each batch was found to be 167; extremes: minimum 128, maximum 252. Immediately after hatching out, the larvae were caught and put in small flasks with a bit of mud in the bottom (1 to 10 larvae per flask). In the water we placed a few previously soaked stalks and some freshly dried meadow grass; a day or two later we added fresh green confervoid water-plants and a few drops of hay infusion. The larvae of this species feed mainly on periphyton; they do not eat tender green confervoid water-plants as much as do the larvae of *C. bitaeniorhynchus* or *Aë. toget*.

We observed quite a high mortality of the larvae in the experiments: the least mortality (37%) was at temperature 20-25°C.

The emergence of the larvae from each batch of eggs was completed as a rule within 24 hours; in three or four days in a few cases only. The mean duration of the development of each stage, at different temperatures and under optimum conditions of feeding, is shown in Table 1.

Table 1. Duration of development (in days) of *C. tritaeniorhynchus* at constant temperatures

Water Temperature	Maturation Period of eggs in the mosquito	Duration of Water Stages							Period from imago to imago
		Egg stage	L _I	L _{II}	L _{III}	L _{IV}	P (Pupa)	Total	
17°C	9	4	5.5	6.5	6.5	6	4	32.5	41.5
19-20.5°	9	3	4.5	5.5	5	4.5	3	25.5	34.5
24-25°	5	2	3.5	3.5	3.5	3	2	15.5	22.5
28-30°	4.5	1.5	2.5	3	2.5	3	2	14.5	19

Development was observed to be accelerated with rise of temperature in all experiments conducted under conditions of adequate alimentation. When feeding was inadequate (only a few drops of hay infusion given) the development period at all temperatures was considerably longer. The variation between the two extremes of duration was insignificant for every water stage except the egg stage; it did not exceed $1\frac{1}{2}$ days or in very rare instances 2 days. The duration of the egg stage at any one temperature fluctuated over a very wide range, particularly at temperatures higher than 20°C . In most experiments at these temperatures the duration of the egg stage was 1 to $1\frac{1}{2}$ days. At temperatures $24-25^{\circ}\text{C}$ and $26-30^{\circ}\text{C}$, a mass hatching-out of the larvae sometimes began less than 24 hours after the eggs were laid. The variation in the length of the egg-stage at these temperatures was seen to be 4 or 5 days; at a temperature of 17°C , it was 19 to 20 days, and at 5° it was 2 to 3 days. At all temperatures however the duration of the egg stage was near to the mean in the great majority of cases.

The optimum temperature for the development of the water stages of *C. tritaeniorhynchus* in our experiments lay between the limits $20-25^{\circ}\text{C}$, for at this temperature (as already been stated, supra) the mortality of the larvae was at a minimum, and the viability of the adult form at a maximum. On the other hand, even a temperature of $28-30^{\circ}\text{C}$ must be regarded as already quite detrimental to them, notwithstanding the shorter development period.

At a temperature of $9-9.5^{\circ}\text{C}$, the activity of the larvae, as far as their movements are concerned, was greatly reduced, and at $8-7.5^{\circ}\text{C}$ they remained motionless on the bottom for the greater part of the time and only the roughest kind of stimulation could get them out of this numbed state (prodding, violent shaking). At a temperature of $3.5-3^{\circ}\text{C}$ all the larvae in the experiment died.

The lowest temperature at which moulting was observed was $+9^{\circ}\text{C}$. We may justifiably take this temperature as the lower threshold for the development of the larvae of this mosquito.

Gonotrophic dissociation in *C. tritaeniorhynchus* was already observed by the first half of September. We were unable to secure ovipositing by females caught under natural conditions at the end of August and during the first few days of September, but in the case of females caught in mid-September we secured ovipositing in isolated cases only. Upon dissection of the females which did not oviposit during a considerable period after feeding, we found a strong development of the fat body, and we noted development of the ovaries to phases II and III. During this period no ovipositing was as a rule observed at temperatures lower than $15-17^{\circ}\text{C}$, while in isolated cases it did not take place even at temperatures higher than 20°C . This, it seems, may be explained by the greater part of the *C. tritaeniorhynchus* population passing into the diapause state.

Observations made by us on the duration of the developmental period in *O. tritaeniorhynchus* with the temperature varying in different ways did not produce any noticeable speeding up as compared to development at an average temperature.

2) *Aë. (F.) japonicus*

This species is considerably more difficult to rear in the laboratory than *Aëdes (F.) togoi*. For our experiments, we used larvae and batches of eggs from natural and artificial bodies of water, also adults both caught in their natural environment and reared in the laboratory.

Under laboratory conditions the females of *Aëdes (F.) japonicus*, after a fairly long stay (10 days) in cages of large size, laid fertile eggs in a small percentage of cases.

In cages with males, the females fed on white mice and guinea pigs, which they attacked quite avidly and then settled to make individual layings of eggs. In the great majority of the experiments a single blood-meal was sufficient for the maturation of eggs. Here, as in the case of *Aë. (F.) togoi* (CHAGIN), the number of eggs laid varies considerably: the average number in our experiments was 52, with extremes 11 and 151. The number is directly proportional to the amount of blood ingested by the female, as noted by DETINOVA in connection with other species of *Aëdes*.

However, in some experiments departures from this high degree of gonotrophic harmony were observed (BEKLEMYSHEV). In five experiments, females after a single blood-meal did not lay eggs. Four of these cases occurred at 22-26°C and one at 15-21°C. At the temperature of 22-26°C no eggs had been laid eleven days (in one case) or 14 days (in 3 cases) after a single blood-meal; after a second ingestion of blood, laying was observed on the 7, 8, 9, and 10th days. At a temperature of 15-21°C no eggs had been laid 14 days after the single blood meal; after a second ingestion of blood laying took place on the 14th day. In the case of females kept on sugar syrup, we did not observe development of the ovaries beyond phases II-III.

Aë. (F.) japonicus, as in the case of *Aë. (F.) togoi*, lays her eggs on the surface of a substrate and in contact with the water; in the process the eggs are stuck firmly to the substrate and for the most part are arranged linearly and perpendicular to the surface of the water. Ovipositing directly on the surface of the water was observed in isolated cases.

After hatching, the larvae were immediately caught and put into flasks of water, 1 to 10 individuals together. The flasks had a little mud on the bottom, along with two or three old

leaves and bits of thin dry twig. In addition, a small amount of fresh green hairweed [*Conferna*] was added a day or two later. The larval feeding depends mainly on periphyton, but under the experimental conditions they fed very well on hairweed.

The larvae are exceedingly shy: on the slightest disturbance they drop quickly to the bottom and take cover either under litter (a leaf or splinter) or in the upper layers of the mud. For the most part they remain in the darkest sections of the flask. In the case of this species, the emergence of the larvae from any one batch was never observed to be such a long-drawn-out process as in the case of *Aë. (F.) togot* (CHAGIN). The hatching of the larvae was as a rule completed in two or three days time, and only in a few cases did it extend to 7 or 8 days.

The duration of the development of each stage, at various temperatures, is shown in Table 2.

Table 2. Duration of development (in days) of *Aë. (F.) japonicus* at different temperatures

Water temperature	Maturation period of eggs in the mosquito	Duration of Water Stages							Period from imago to imago
		Egg stage	L _I	L _{II}	L _{III}	L _{IV}	P (Pupa)	Total	
17-18°	9	7	3.5	4.5	4.5	5	4	26.5	37.5
20-21°	8	6	2.5	3	3	4	4	22.5	30.5
24-26°	7	6	2	2	2.5	3	2.5	18	25
29-32°	8.5	5.5	1.5	1.5	Observation unsuccessful because of mass mortality of larvae.				

The least mortality of larvae (23%) was seen at 20-21°C, and the largest and most viable insects reached the adult stage at temperatures 20-21° and 24-26°C; in our experiments, these were the optimum temperatures of development of the water stages of *Aë. (F.) japonicus*. The variation between the extremes of the development period for each stage at the above mentioned temperatures did not exceed 1 to 1½ days. At 29-30°C, we observed a very short duration in the first larval phases, but not a single larva reached the stage of pupation.

This is reason for regarding a steady temperature of 30-32°C as the upper threshold for development of the water stages of *Aë. (F.) japonicus*. We were unable to establish a lower threshold for the development of this species under laboratory conditions; development of larvae instars III and IV, and of pupae, was observed at 9-11°C; the mean duration of each of these

instars at the said temperature was 11.5 days; that of the pupal stage was 7 days; the extremes were 7.5 and 14 days for the larvae and 5-11 days for the pupae.

With the temperature varying, the duration of the development period was somewhat longer than in the case of the corresponding constant mean temperature.

With both constant and varying temperature, there were considerable variations in the period of maturation of the eggs in the mosquito: the maximum periods were usually one and a half to two times the minimum, while the greatest duration of the maturation period was observed when the amount of blood ingested by the female was least. We also observed the mosquitoes to attack vigorously both with ingested blood still remaining from previous meals and also shortly before the laying of eggs. This fact, together with the above mentioned cases of the eggs maturing only after a repetition of the blood intake, gives us grounds for supposing that repeated ingestions of blood occur fairly frequently under natural conditions in the case of *Aë. (F.) japonicus*.

3) *Aë. esoënis*

Aë. esoënis is quite difficult to rear under laboratory conditions. It is very sensitive to pollution of the water by rotting organic material.

We did not observe copulation of this species in captivity. Females caught in the natural environment were used in the tests. In the laboratory they were fed, like the previous species, from white mice and guinea pigs.

In all the tests, ovipositing took place after a single blood-meal. As in the cases of *Aë. togot* and *Aë. japonicus*, this species always showed a relationship between the number of eggs matured and the quantity of blood ingested by the female. The average number of eggs laid in a batch was, in our experiments, 73, and the extremes were 16 and 118.

After a single copulation, the female is capable of producing a number of fertile batches of eggs. In one experiment, within a period of 23 days a female of *Aë. esoënis*, caught in the natural environment, gave four layings of fertile eggs without further copulation. She was given a new blood meal from a white mouse 1 - 2 days after each laying. In the four batches she deposited a total of 334 eggs, which hatched out larvae. After the fifth meal on the 12th day, she did not again oviposit, and finally died: the ovaries were found to have degenerated.

When females of this species were kept on sugar syrup, we never once succeeded in getting eggs from them.

Aë. esocænis oviposits on the surface of water. In isolated experiments, we observed batches of eggs to be laid on the surface of a substrate and contiguous with the water. The eggs were not firmly attached to this substrate. The hatching of the larvae from any single batch was in the majority of experiments completed in a period of 3-10 days.

In five experiments conducted at 18-22° and 22-26°C, a considerable number of eggs still remained after the initial emergence of larvae over a period of 1-2 days; no further hatching of these eggs took place in a 17-23 day period. At the end of this time a number of eggs were opened and found to contain fully formed, live larvae. After this the remaining eggs were placed, 10 or 15 together, in water at various pH values (pH = 5.0-8.2); during the following day larvae were seen to emerge from part of the eggs in water at pH values 6, 6.6, 6.9, 7.9, 7.5, and 8.2. Only in the initial test (pH = 7.2) and in the test at pH = 5.0 did larvae fail to emerge.

Twenty-eight days later, the few eggs left were opened, and in them, as before, fully formed live larvae were found. Only in the test at pH = 5.0 did we find the larval embryos to have perished.

No further hatching of larvae was induced, neither by a short period of warming of the remaining eggs to temperature 36-38°C, nor by cooling the whole body of water to the freezing point, nor by changing its pH. So far, we have not studied the cause of the phenomenon described.

After hatching, the larvae were kept, 1 to 5 individuals together, in flasks, on the bottom of which there was a small quantity of mud and some bits of old leaf; in addition, two or three short stalks of dried grass were dropped into the water.

The larvae of *Aë. esocænis* feed on periphyton and seston*; they do not eat hairweed, but a little of it added to the water increases the survival powers of the larvae. The average duration of the developmental period in *Aë. esocænis* at different temperatures is given in Table 3.

* Very small plankton retained only by the finest nets.

Table 3. Mean duration of development cycle (in days) in *Aë. aëoënsis* at different temperatures

Water temperature	Maturation period of eggs in the mosquito	Duration of Water Stages							Period from imago to imago
		Egg stage	L _I	L _{II}	L _{III}	L _{IV}	P (Pupa)	Total	
15-16°	?	10	4	5	5	4.5	4	32.5	?
29-30°	7	8.5	2.5	3	3	3.5	3	23.5	31.5
24-25°	5.5	6.5	2.5	2.5	2	3	2	18.5	25
28-30°	5	8.5	2.5	2	1.5	2	1.5	18	23
32°	?	?	3.5	3	4	Larvae die.			

The least mortality of larvae (42%) and the greatest viability of the mosquitoes was observed at 19-20°C; temperatures in the range 18-22°C were the optimum for the development of the water stages of *Aë. aëoënsis*.

The difference between the extremes in the length of the developmental period for each stage was least in the experiments at 19-20° and 24-25°C; it did not exceed 1 to 1½ days, or in very rare cases 2 days. At 28-30°C, the developmental period of each stage fluctuated considerably in length; both shortening and lengthening of the period were observed. The larvae at these temperatures were less active, and their mortality was as much as 89%. The mean duration of the egg stage at this temperature was greater than at 24-25°C, the developmental process in some of the experiments dragging out to 14 days. At 32°C, all the larvae perished in the first to third instars. In some cases the development of the larvae in each of the periods of growth dragged out for six to eight days. The reaction of the larvae to stimulation was sluggish. In our experiments, this temperature was the upper threshold for the development of the stages of *Aë. aëoënsis*.

Observations carried out with the temperature varying in different ways showed that in cases where the upper limit of the variation came within the zone of detrimental temperatures, the development of some stages had a greater duration than in those cases where the upper temperature limit was below this level. This was particularly well marked in the egg stage. Thus in experiments with the temperature fluctuating between 18 and 28°C, the mean duration of the developmental period of all the water stages was equal to 25 days, while the egg stage was on the average completed in 11½ days (extremes, 5 and 17 days).

With the temperature varying within limits lower than the detrimental level, development was completed in periods equal to or somewhat less than at the corresponding mean temperature.

II. Observations in Natural Surroundings

Observations under natural conditions showed that *C. tritaeniorhynchus* by the nature of its phenology is, under the climatic conditions of the Maritime Territory, a late-summer species. Its first larvae, usually in the second or third instars, are to be found on the end of the first to the beginning of the second half of July. On this basis, we must take the end of the first half of July as the time when the females of *C. tritaeniorhynchus* is so restricted that over the six-year period of our observations we did not once succeed in detecting them among mosquitoes attacking. P.A. PETRISHCHEVA remarks that during the said season of the year they constitute not more than 0.01% of the total number of attacking mosquitoes.

The hatching out of the first generation takes place exclusively on bodies of water which are entirely typical and which may be described either as permanent or long in existence (lake-shore or river-bank swamp, rice-fields, permanent swampy patches in hollows). The extent of the initial distribution of the mosquito population is determined by the number of typical hatching-ponds and their distribution over the terrain of a given district at the time when the hibernating mosquitoes take wing. Observations carried out over a number of years testify to the fact that *C. tritaeniorhynchus* in the southern areas of the Maritime Territory is capable of producing three generations per season, of which the second generation is the most numerous.

The first generation of mosquitoes begins to get its wings at the end of July, and this process is complete at the end of the first ten days of August.

During this period of the year (water temperature 16-27°C), the mean duration of development of the water stages, as determined by field observations, is 19 days, while the whole cycle from imago to imago takes 23 days. Consequently, in the Maritime Territory, the second generation had reached the adult stage by the first few days of September.

This period must also, as indicated above, be taken as that of the appearance of the first females to enter the state of gonotrophic dissociation. But not all the females are in this state; part of them are still capable of ovipositing. Hence in the first half of September we still observe the odd batch of eggs to be laid, and the development of another generation from them.

The third, not very numerous generation of *C. tritaeniorhynchus* reaches the adult stage by the 5th to the 10th of October. Isolated larvae of this generation can be found up to the second half of October.

Thus, in the Maritime Territory, the whole season of active life of *C. tritaeniorhynous* subdivides into the following four periods:-

1) Hibernating females take wing. Second half of June to first few days of July.

2) Period of initial growth of mosquito population. July to first ten days of August.

3) Period of wide distribution of population and its further increase to the maximum point. From the first few days of August to the end of the first 10-day period of September.

4) Period of sharp decline in the active population, ending in the complete cessation of activity of the winged form. From the middle ten days of September to the first few days of October.

Aë. esoënts is an early-summer species: in the Maritime Territory it reaches its maximum numbers in June and the first half of July.

The first larvae of this species were found by us in the last few days of April (minimum atmospheric temperature averaging +2.5°C), while the first generation was observed to get its wings on the 18th to 20th of May (in 1944). The mean atmospheric temperature over the period of development of the water stages was within the limits 11-14.5°C. During this period, larvae of the species were to be found in puddles formed in the meadows by accumulations of melt-water.

The maximum population of this species is determined by the first generation taking flight, and is observed from the 15th of June to the 5th or 8th of July. From the end of the first half of June to the end of August the larvae of the species are found in very insignificant numbers, a fact which may be explained not only by the drying up of the greater part of the typical water-bodies but also, it seems to us, by the unfavorable temperature-conditions in the summer period. This is supported by the fact that we find the odd larva only in heavily shaded, relatively cool water. In the first days of September the larvae of *Aë. esoënts* were to be found, in the southern areas of the Maritime Country, both in newly formed and in previously existing bodies of water. Their relative numerical strength in the first half of September amounted to 7.5-8.2% in typical ponds.

All this gives grounds for believing that in the Maritime Territory *Aë. esoënts* produces a mass flight of only one generation; adulthood is reached by the second generation, and possibly in some places by a third generation, in a process which is spread out over the whole length of the season. These generations are insignificant as far as the number of mosquitoes

reaching the adult stage is concerned; they are incapable either of maintaining the population through the whole summer season or of causing any marked increase at the beginning of autumn.

Only in years with regular and abundant precipitation did we observe a less marked decline in the population of this species in June; this is to be explained by a greater simultaneity in the maturing of second-generation adults in these years.

Aē. (F.) japonicus. Larvae of the third and fourth instars were recorded in 1914 on the 22nd-23rd of May. Emergence of adults of the first generation began on the 28th-29th of May. The minimum atmospheric temperature for the first half of May, in the district where we detected this species, was within the limits 1.8-7°C and the mean temperature was within the limits 7-17.4°C. The chances of subsequent generations of this species developing is in great part determined by the existence of typical hatching pools, small artificial and natural bodies of water, within the area which is its habitat. In the autumn period we were able to find *Aē. (F.) japonicus*, both in the adult and larval forms, until the end of September and the first days of October.

Under favorable meteorological conditions (scanty precipitation) there may be a break in the uninterrupted transition from one generation to another; this was observed in 1944 at a number of points in Vladivostok District. Under favorable conditions, however, *Aē. (F.) japonicus* in the Maritime Territory may produce a flight of not more than four generations, which will reach its numerical maximum at the end of the second half of June. The number of generations in one season may differ at different places, this depending on the way the various conditions for the hatching of this species fall into combination at any place.

On the basis of the above facts, control measures against the larvae of *C. tritaeniorhynous* in the areas where this species is present in large numbers should start at the end of the first half of July and terminate in the second half of September; in places where *Aē. esoenis* and *Aē. (F.) japonicus* are widespread, such measures should extend from the end of the first half of May to the beginning of the second half of September.

The thoroughness of anti-larval measures to be taken at different periods of the season must be decided in each district on the basis of larva-reconnaissances.